

Microbiological Tests Performed During the Design of the International Space Station Environmental Control and Life Support Systems. Part 1, Bulk Phase

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The design and manufacturing of the main Environmental Control and Life Support Systems (ECLSS) for the United States segments of the International Space Station (ISS) was an involved process that started in the mid 1980's, with the assessment and testing of competing technologies that could be used to clean the air and recycle water. It culminated in 2009 with the delivery and successful activation of the Water Recovery System (WRS) water processor (WP). The ECLSS required the work of a team of engineers and scientist working together to develop systems that could clean and/or recycle human metabolic loads to maintain a clean atmosphere and provide the crew clean water. One of the main goals of the ECLSS is to minimize the time spent by the crew worrying about vital resources not available in the vacuum of space, which allows them to spend most of their time learning to live in a microgravity environment many miles from the comforts of Earth and working on science experiments. Microorganisms are a significant part of the human body as well as part of the environment that we live in. Therefore, the ISS ECLSS design had to take into account the effect microorganisms have on the quality of stored water and wastewater, as well as that of the air systems. Hardware performance issues impacted by the accumulation of biofilm and/or microbiologically influenced corrosion were also studied during the ECLSS development stages. Many of the tests that were performed had to take into account the unique aspects of a microgravity environment as well as the challenge of understanding how to design systems that could not be sterilized or maintained in a sterile state. This paper will summarize the work of several studies that were performed to assess the impacts and/or to minimize the effects of microorganisms in open, semi-closed and closed loop life support system. The biofilm and biodeterioration studies that were performed during the design and test periods will be presented in a future publication.

Nomenclature

CFU	= Colony Forming Units
ECLSS	=Environmental Control and Life Support Systems
EEF	=End-Use Equipment Facility
ISS	= International Space Station
MCV	=Microbial Check Valve
mL	=Milliliters
PCWQM	=Process Control Water Quality Monitor

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SSF	=Space Station Freedom
ST	=Stoarge Tank
WP	= Water Processor
WRT	= Water Recovery Test
WRS	= Water Recovery System

I. Introduction

Microorganisms are ubiquitous contaminants of most aqueous-based fluid-handling systems. In the absence of antagonistic environmental conditions or chemical agent, bacteria, fungi, and protozoa can utilize a wide range of energy sources for growth. Bacteria, in particular, are well-adapted to survival in a variety of environments, including those that are considered oligotrophic (Marshall, 1988). Fungal contaminants are can also be present as contaminants in water systems, although they are less well-adapted to wide-ranges of environmental physicochemical conditions. Free-living protozoa are associated with contaminated water systems, and can harbor pathogenic bacteria as endosymbionts (Abbaszadegan, et al., 1997; Donlan, et al. 2005). Viruses are obligate intracellular pathogens, but can be recovered from wastewaters and potable waters contaminated with human or animal waste (Gerba and Rose, 1990).

Understanding the microbial ecology of water systems is an important factor in assessing both the health-risk to crew and the potential for biological fouling. Potable water meeting U.S. EPA requirements (U.S. EPA, 2003) used for drinking, bathing, and washing is not sterile, but is free or microorganisms of sanitary significance. However, many of microorganisms that are part of what is considered to be the otherwise non-pathogenic flora of potable water can be a health risk for space travelers (La Duc, et al., 2004). Therefore, characterizing the microbiological community in various fluid handling systems—where crew contact is possible—is essential to assessing human health risk.

In addition to the potential health risks associated with water systems bioburden, microbial growth and activity can result in biological fouling of critical operational systems. Bulk phase bacteria can attach to surfaces, produce extracellular polymeric substances (EPS), entrain organic and metallic bulk phase contaminants, and colonize various engineered materials as biofilms (Mittelman, 1998). Biofilm formation can lead to mechanical blockages, reductions in heat transfer efficiency, and microbially influenced corrosion. Control of both bulk phase and biofilm populations is challenging in space environments, where treatment options are constrained by safety, materials compatibility, and weight considerations.

The role of bulk phase microbial contaminants, bacterial biofilms, microbially influenced corrosion, and biofouling treatments in International Space Station (ISS) ECLSS operations was a focus of several studies from 1990-2009 that were carried out by NASA or their collaborators. In this communication, we summarize the bulk phase community structure and contaminant level studies that were carried out during this timeframe. A series of related studies, involving biofilms and biofouling control strategies, were also conducted. These studies will be described in a future communication.

II. The Environmental Control and Life Support Systems Water Recovery Test

The main goal of the Phase III Water Recovery Test (WRT) program at NASA/Marshall Space Flight Center (MSFC) was to design, develop and test a physical/chemical water system for the Space Station, that would recycle water from various human and equipment sources. When the program was started in the late 1980's, the hardware was intended for use in the Space Station Freedom (SSF) and the water system was designed with 2 loops: one to recycle water from hygiene activities and urine for use as hygiene water and one to recycle water from the humidity condensate for potable water. The Phase 3 WRT comprised several stages of testing, each one with the incorporation of lessons learned from the previous ones and eventually with the incorporation of the big design change that was the result of the changes that took the SSF to what is today, the ISS.

The first set of tests was the WRT Stages 1A, 2A, and 3A. It was a 2-loop design, with hygiene and potable water recycled using different subsystems for water recycling in each loop, such as the Thermoelectrically Integrated Membrane Evaporation System (TIMES) as the urine processor, an Ultrafiltration (UF)/ Reverse Osmosis (RO) in the hygiene water loop (Figure 1) and a Multifiltration (MF) system in the potable loop (Figure 2). The potable loop recovered and purified water for drinking purpose from humidity condensate derived from respiration, perspiration and equipment emissions. The hygiene loop recovered and reclaimed water from urine, shower/handwash, dishwashing and clothes wash. Stages 1A, 2A and 3A were completed in July of 1990. Although these first tests were generally successful and the main objectives of operating a partially closed hygiene loop and an open-potable loop were met, hardware performance challenges were identified, some related to facility issues. All microbiological and chemical water quality parameters, with the exception of Total Organic Carbon (TOC), were generally within specifications in the reclaimed potable and hygiene waters (TIM 103564).

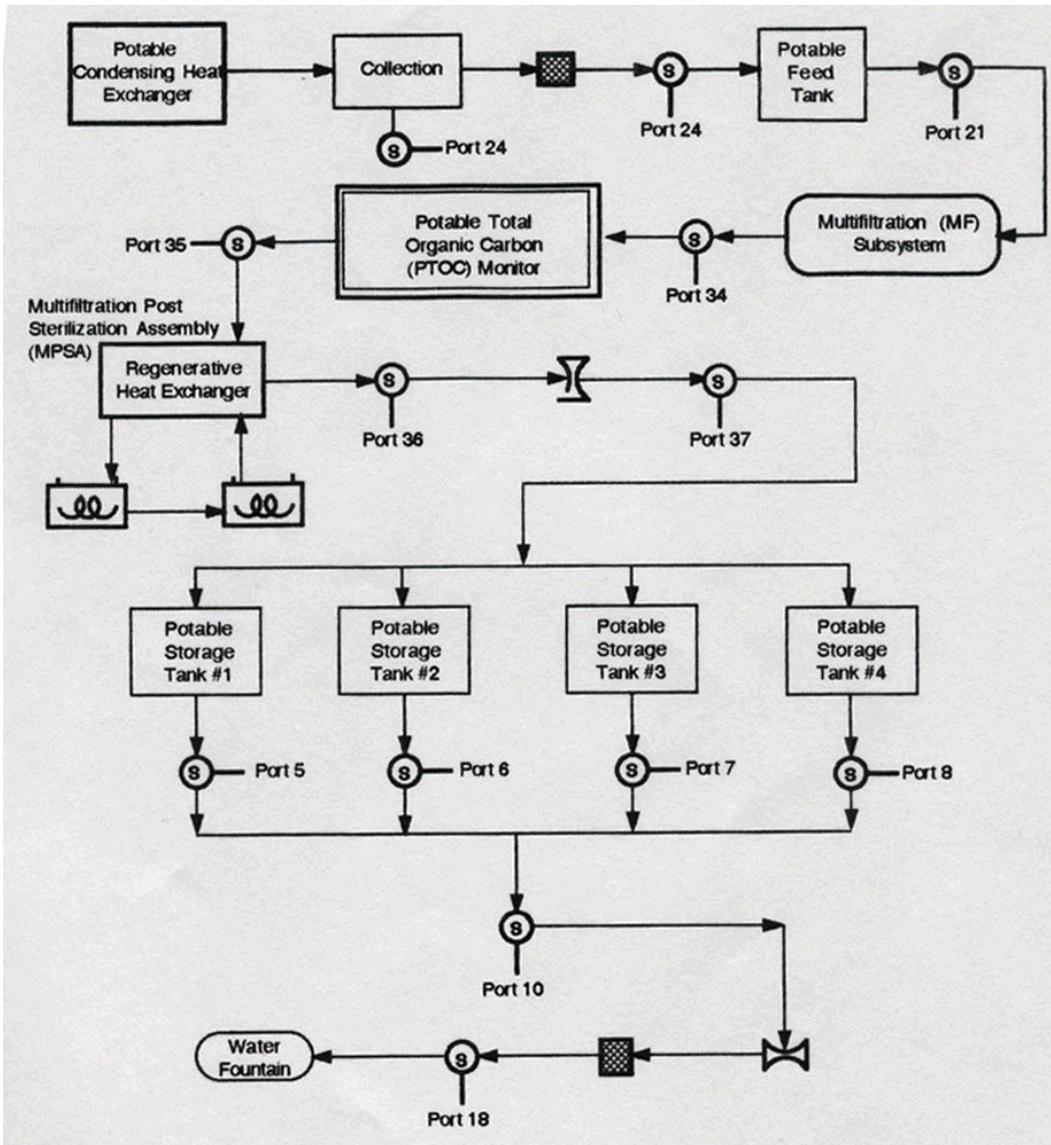
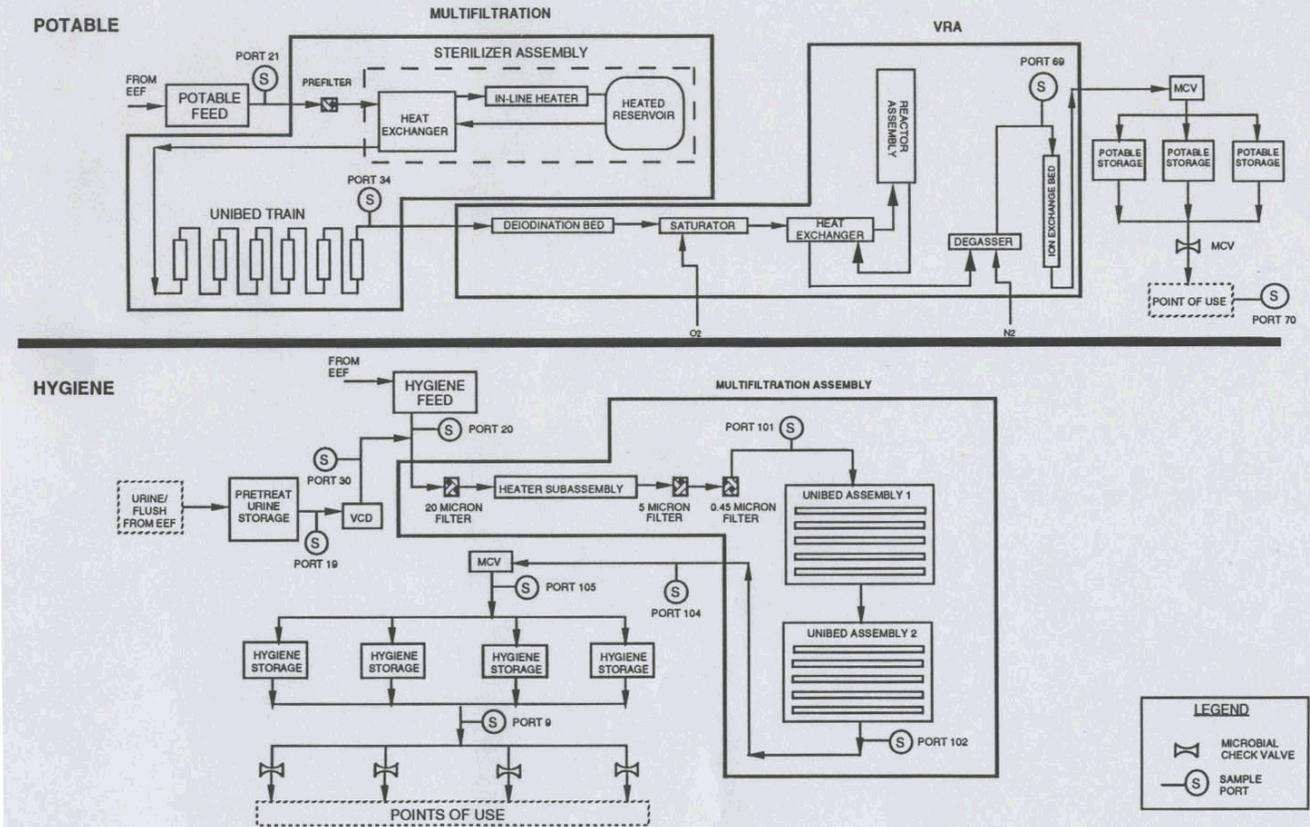


FIGURE 2. WRT Stage 1A, 2A and 3A Potable Water Processing Schematic



In mid-1991, the next set of tests was performed, WRT Stage 4/5. This system was also a 2-loop design, one for hygiene and one for potable water. For Stages 4/5 hygiene loop, the urine processor baselined technology was selected to be the Vapor Compression Distillation (VCD), instead of the TIMES, and MF beds, which incorporated a series of ion exchange resins and organic adsorbents similar to the potable reclamation system. At this time, the dishwasher was removed from the test because it had been deleted from the SSF program in September of 1990. This test marked the first in WRT that was operated in recipient mode, wherein test subjects used water reclaimed from shower, handwash, and laundry and tasted water reclaimed from humidity condensate. Reclaimed potable and hygiene water routinely met the water quality specifications and was considered acceptable by the test subjects for hygiene use and drinking water (ICES 1992).

FIGURE 3. WRT Stage 4/5 Potable and Hygiene Water Processing Schematic

During the last quarter of 1990, the space station program underwent significant restructuring to meet revised funding constraints. During this exercise, the decision was made to combine the separate ECLSS potable and hygiene water loops into a single-loop system. This change was implemented in the WRT Stage 7 test (completed in February 1992), which verified the feasibility of single-loop operation (TIM 108398). Limited recipient mode testing was also conducted during Stage 7. Water quality specifications were routinely met and product water generated from a combined waste stream was considered acceptable by the test subjects for both hygiene and potable use.

WRT test data indicated that the Water Processor (WP) sterilizer provided minimal effect on the microbial population. The WP in-line pre-sterilizer was not a true “sterilizer” in microbiological terms: the system heated the wastewater up to 250°F for 20 minutes. This “sterilizer” was deleted from the WP design (Figure 4), relying on the multifiltration adsorbents and the Volatile

Removal Assembly (VRA/ catalytic oxidation reactor) to reduce the microbial population to within the ISS water quality specification. This design modification was verified during the WRT Stage 8 test, which showed that MF beds and VRA were capable of meeting the microbial water quality specification. No recipient mode testing was conducted during Stage 8.

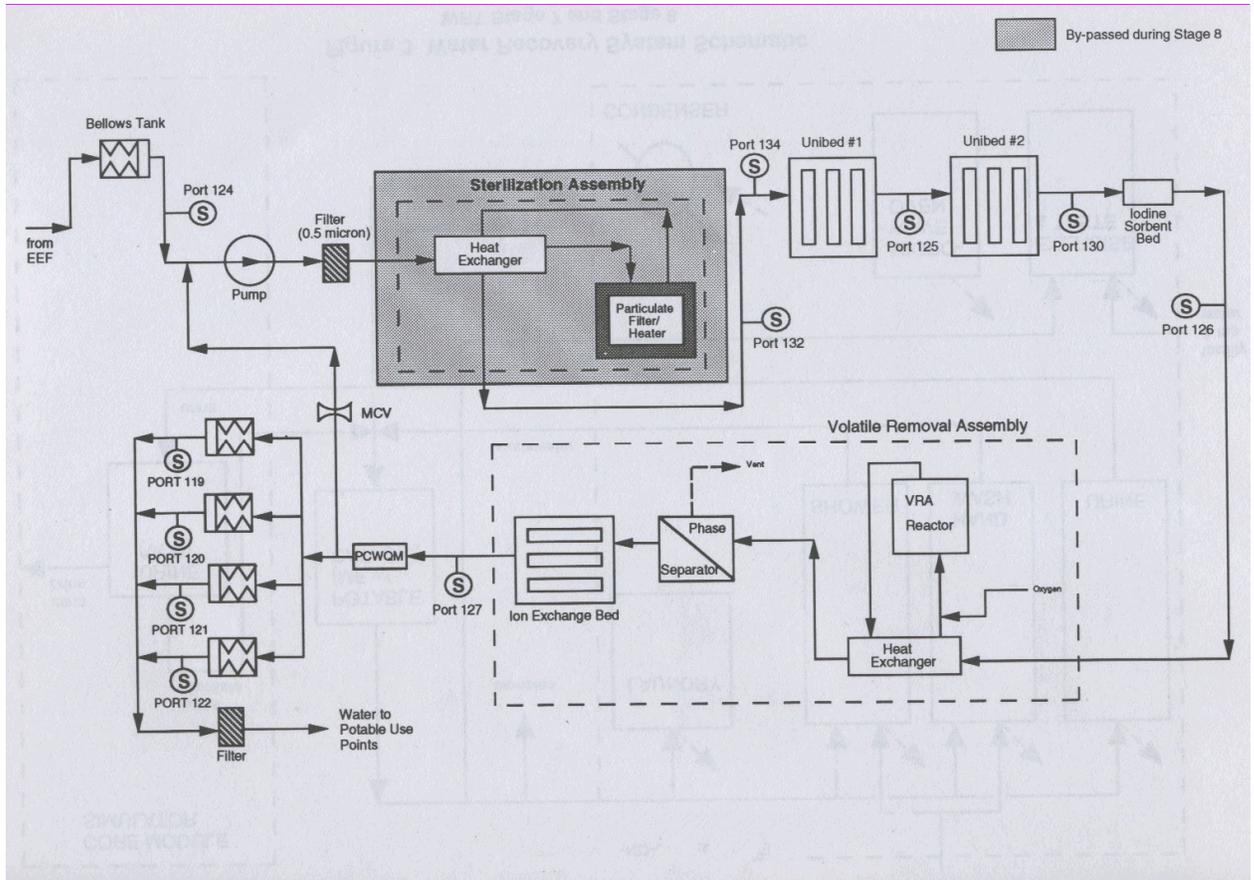


FIGURE 4. WRT Stage 7/8 Water Processing Schematic

In 1993 the Space Station program underwent additional restructuring that resulted in the involvement of the Russian Space Agency (RSA). The conclusion to this effort was the ISS, which replaced the previous program, the SSF. The WRT Stage 9 objective was to operate higher fidelity water recovery hardware integrated to reflect the ISS WRM configuration in an automated system level control scheme (TIM 108498). Though significant information was obtained during WRT Stage 9, performance anomalies occurred that require further investigation. These were addressed in WRT Stage 10, to also be conducted in recipient mode, further addressing issues related to long-term water reuse.

The last stages of the WRT test program were Stages 10 and 11. The goal of WRT Stage 10 was to assess the automated operation of the ISS Water Recovery & Management (WRM) System, including the WP, Process Control Water Quality Monitor (PCWQM), Urine Processor (UP), and Urine Collection System (UCS) assemblies when operated with maximum reuse of reclaimed water. The WRM System processed equivalents of ISS wastewaters; each wastewater was generated in a manner similar to the ISS environment and delivered real-time. Artificial

waste waters were generated as necessary and delivered based on ISS projections at the time (. A simplified functional schematic of the test system for WRT Stage 10/11 is provided in **Figure 5**. Testing was performed over 150 days.

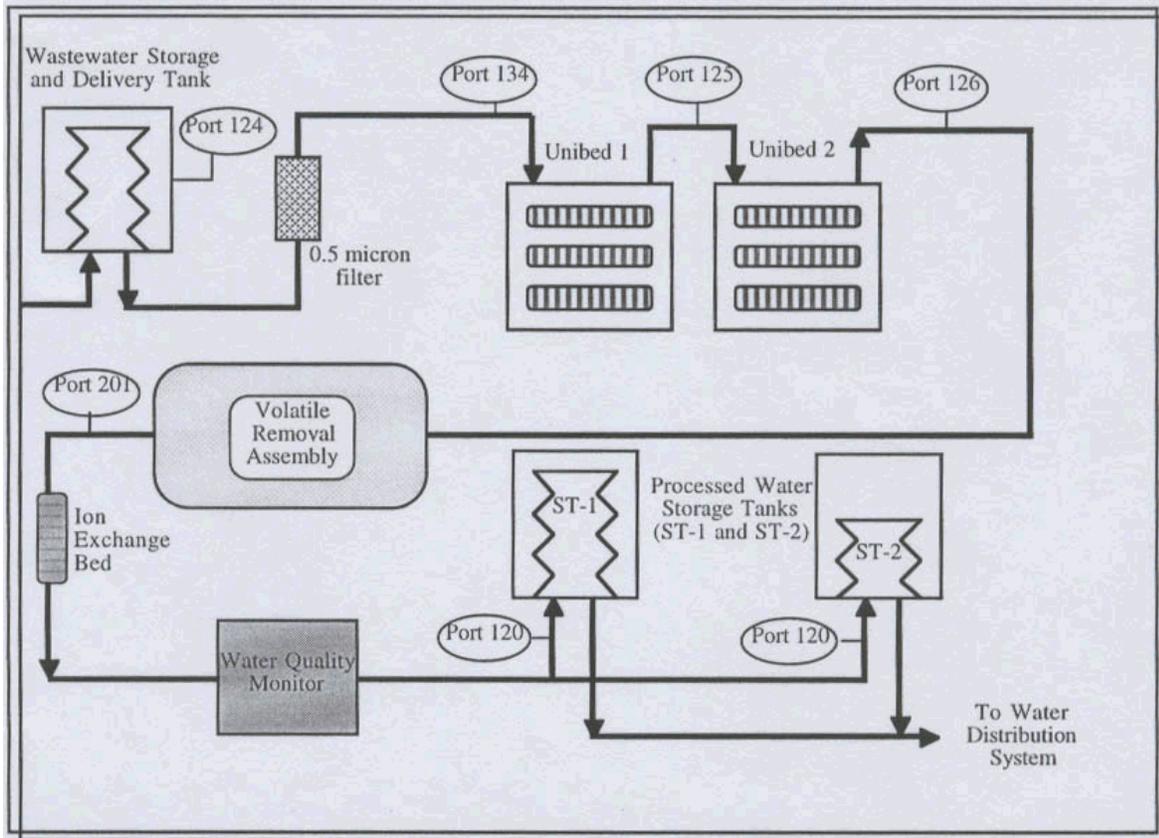


FIGURE 5. WRT Stage 10/11 Water Processing Schematic

III. ISS ECLSS Microbiology Studies, 1990-2009

Developmental testing of Space Station life support systems included significant design variations of hardware and/or configurations that were or were not chosen for the final flight systems. Microbiological data collected during the early years provides information that might be useful in the understanding of current and future system performance issues.

Microbiological analyses/studies were done before, during and after integrated testing, in standalone mode, and in laboratories. This testing provided information on the hardware performance in complex systems at various levels of microbial removal stages, in the absence of microbial input (humans waste and metabolites were used in the test), and in water flowing through stainless steel or titanium tubes. Standalone tests were designed to better understand the particular effects of human generated microbial populations on system configurations (for example tanks, tubes, condensing heat exchanger surfaces). Laboratory tests were designed to

address issues of concern that required a controlled environment and could be performed on a small scale.

The majority of the microbiological tests/analyses performed during the design and testing of the life support systems were related to concerns with the Space Station water recycling system. The Water Recovery Test (WRT) program was designed to demonstrate that integrated subsystems configurations could produce safe water for human consumption. Before the design, development and launch of the ISS Water Processor (WP), all of the water used by the crew during space flights was transported from Earth, or generated in-flight as a by-product of fuel cells.

The methods and media used for the microbial analyses of water samples from the WRT, included (but were not limited to):

- Epifluorescent Direct Microscopic Counts- to rapidly determine the total number (viable or not viable) of microorganisms in the wastewater. Given that the minimum detection limit of the procedure used was $9.5E+04$ cells/100 mL, epifluorescent direct counts could be used for a rapid assessment of recycled product water only.
- R2A culture medium- incubated at 28°C (7 days), for the general enumeration of heterotrophic bacteria in water, particularly bacteria that grow under low nutrient conditions and utilize a variety of carbon sources for energy.
- Chocolate Agar Enriched (CAE)- incubated at 35°C with 5% CO₂ (2 days), for the enumeration of aerotolerant eutrophic mesophiles (AEM) bacteria. These are organisms from normal body flora that may have medical significance.
- Emmon's Medium- incubated at 25°C (5 days) for the recovery of yeast and filamentous fungi. Rose Bengal and chloramphenicol was added to the media to inhibit the growth of bacteria.
- Membrane Fecal Coliform (mFC) media- incubated at 44.5 °C for 24 hours was used for the recovery of gram negative bacteria that live in the intestinal tract of animals and humans.
- Identification: A representative number of each different colony morphology from enrichment and enumeration media was streaked for isolation by the quadrant streak plate method. On each isolate, two serial transfers were performed to ensure the purity of the culture for identification. The MIDI, Biology, and Vitek were used to identify microorganisms based on fatty acid composition, substrate utilization, and biochemical reactions.

The following studies were conducted on the WRT:

1) Microbial characterization of Processed Water- The WRT was designed to assess the performance of the integrated water recovery system to produce water that met required specifications, but performance of individual subsystems was also assessed during testing. Sample ports were located before and after key subsystems to assess the chemical and microbial loads before and after the treatment. Most of those ports were sampled periodically, on an as-needed basis, not as part of the main sample schedule. Overall the water processor was very effective removing the bacterial population from the wastewater; processed water had low

concentration of bacteria and chemicals. It was noticed that bacteria isolated and identified in the samples during the first stages of the WRT were more diverse than the bacteria that was isolated during the last stages of WRT (Stage 10/11). This might be due to the increase in the effectiveness of the process, as the hardware was further developed and optimized. It could have also been due to the increase of awareness of the need to use aseptic techniques during hardware installation and during sampling.

During WRT Stage 1A, 2A and 3A wastewater from the hygiene loop had an average heterotrophic bacterial load of $>7.5 \times 10^6$ CFU/100mL on R2A media. The bacterial concentration was successfully reduced to an average of 3.9×10^0 CFU/100mL. Wastewater from the potable loop had an average heterotrophic bacterial load of $>2.5 \times 10^6$ CFU/100mL on R2A media. The bacterial concentration was successfully reduced to an average of 2.8×10^0 CFU/100mL. *Staphylococcus spp.* were the organism most frequently identified from the clean water samples. Other organisms identified included *Arthrobacter protophormiae*, *Micrococcus spp.*, and *Pseudomonas paucimobilis*

During WRT Stage 4/5 wastewater from the hygiene loop had an average heterotrophic bacterial load of 5.4×10^8 CFU/100mL on R2A media. The bacterial concentration was successfully reduced to an average of 1.3×10^0 CFU/100mL. Wastewater from the potable loop had an average heterotrophic bacterial load of 3.4×10^7 CFU/100mL on R2A media. The bacterial concentration was successfully reduced to an average of 2.3×10^0 CFU/100mL. *Staphylococcus epidermidis* was the organism most frequently identified from the clean water samples. Other organisms identified included *Micrococcus spp.*, *Flavobacter indologenes*, *Acinetobacter spp.*, *Corynebacterium kutscheri* and *Methylobacterium radiotolerans*.

During WRT Stage 7/8 wastewater had an average heterotrophic bacterial load (on R2A media) of 1.1×10^8 CFU/100mL (Stage 7) and 3.5×10^9 CFU/100mL (Stage 8). The bacterial concentration was successfully reduced to an average of 5.0×10^{-1} CFU/100mL (Stage 7) and 1.3×10^0 CFU/100mL (Stage 8). *Staphylococcus spp.* was the predominant organism identified in the processed water in both stages.

2) Viral survival test- During WRT Stage 9, the WP was challenged with a known concentration of viral particles mixed with human generated wastewater. The viruses used for this study (bacteriophages MS2, T-1, VD13 and 23356-B1) were chosen for this study because they behave similarly to the human viruses that could be found in the space station wastewater. Because these viruses are not considered pathogenic to humans, no special safety precautions were needed during the test. The test protocol was prepared by Dr. Christon Hurst from the Drinking Water Research Division of the United States Environmental Protection Agency (USEPA) and NASA personnel. In addition, his laboratory performed the microbiological analysis necessary to assess the quality of the water after the test was completed.

For the viral challenge test, a minimum of 10^7 plaque forming units per milliliter (PFU/mL) per each virus type were mixed with human generated wastewater (90 lbs per batch) and processed in the WP. The wastewater used for this test was a combination of water containing human

metabolic waste from humidity condensate, shower, handwash, urine distillate, oral hygiene and wet shaving. In addition, it contained fuel cell water, a laboratory prepared solution of simulated animal humidity condensate, and equipment off-gassing contaminants

The test was performed as planned and no anomalies with the WP were recorded during the test. No viruses were detected in any of the sample ports after passing the 2 series of MF beds. The overall estimates of viral removal by the first MF bed were 4.8 log₁₀ units for MS2, >7.9 log₁₀ units for T1, >7.7 log₁₀ units for VD13 and >6.1 log₁₀ units for 23356-B1. The overall estimates for viral removal by 2 MF beds in series were >8.6 log₁₀ units for MS2, >8.2 log₁₀ units for T1, >7.7 log₁₀ units for VD13 and >7.2 log₁₀ units for 23356-B1. There are 3 main mechanisms that can be responsible for the viral removal in the MF beds: 1) size filtration; 2) adsorption to the surface of the resins; 3) inactivation by iodine or some other chemical compound on the surface of the resins. From the results of the test, filtration was ruled out as the factor responsible for the viral removal.

Because the viral population challenge was completely removed after the 2nd MF bed, the VRA was not challenged with viruses during the test performed after Stage 9; a second test was performed after the completion of WRT Stage 10. It was performed in 5 days also, using the same type of inoculums used for the Stage 9 viral test, but the viruses were added after the MF beds, prior to entering the catalytic oxidation reactor. Results from that test showed that the VRA has an overall estimated viral removal capability of 6 log₁₀ units. This test confirmed that any viral particle that is not removed and/or deactivated in the MF beds, will be deactivated in the VRA.

The concentration of viruses used for this test significantly exceeded the viral population expected to be found in the ISS wastewater. The results of this viral challenge demonstrate that the WP has an excellent capacity for reducing the disease hazards posed by viruses in the wastewater.

4) Water storage (in tanks)- After the completion of 3 of the WRT stages (Stage 4/5, 7 and 8), processed water was left in storage tanks to assess the potential of microbial regrowth in the recycle water. These tests were done as a result of a concern that the initially viable-but-not-culturable bacteria in the water could multiply in the storage tanks, if the water remained stagnant for long periods of time. Results of these tests are discussed below.

WRT Stage 5- iodinated processed water from the potable and hygiene loop was stored in the tanks for approximately 18 days. The objective of this post-test study was to assess the microbial population and iodine concentration in stored processed water. Water samples were periodically analyzed for heterotrophic bacteria using R2A media and AEM bacteria using CAE media. Residual iodine, iodide, and total iodine concentrations were also monitored.

Potable tank 1 was used to store the process water for the water degradation test. A water sample withdrawn prior to the start of the water degradation study (test day 21), contained <1 CFU/100 mL of bacteria on CAE and R2A media. The amount of Total Organic Carbon (TOC) reported in the water at this time was 0.34 mg/L. Turbidity was 0.1 NTU, iodine residual was 1.33 mg/L,

and pH was approximately 7.0 pH units. The TOC, turbidity, and pH analyses were not performed after test day 21.

The first microbial sample withdrawn from the water tank was on test day 25. The sample contained a microbial load of $1.35E+04$ CFU/100 mL on CAE and 2.00×10^3 CFU/100 mL on R2A. An average microbial load of 1.0 CFU/100 mL on R2A and CAE was maintained by the potable processed water for 11 consecutive days (test day 28 through 38).

The organisms identified on test day 25 were *Comamonas acidovorans* and *Xanthomonas maltophilia*. They were not identified in any of the other water samples analyzed throughout the WRT Stages 4/5 or during the water degradation test. It is possible that this sample was contaminated during sampling and/or processing. *C. acidovorans* and *X. maltophilia* are considered to be ubiquitous in nature and, thus, are likely to be found as contaminants.

Staphylococcus epidermidis was the organism most frequently identified from the water samples (6 out of 13 samples that were taken from the potable tank). Other microorganisms identified during this test included *Micrococcus spp.*, *Flavobacter indologenes*, *Acinetobacter spp.*, *Corynebacterium kutscheri*, and *Methylobacterium radiotolerans*. Most of the identified microorganisms are normally found in water environments and are generally not considered pathogenic to humans. Most of the bacteria found during the water degradation test have been identified during previous testing.

Iodine concentration remained constant throughout this test. Iodine residual was maintained between 1.05 mg/L and 1.47 mg/L and the iodide concentration averaged 0.93 mg/L ranging from 0.52 to 1.22 mg/L. No changes in the iodine/iodide ratio were reported from potable water samples.

Iodine effectively controlled the heterotrophic and AEM bacterial populations in the potable water tank for a minimum of 11 consecutive days. The microbial population was maintained at an average of <1 CFU/100 mL. The microbial count reported for test day 3 (1.0×10^3 CFU/100 mL) was considered contamination (likely during the sampling) because the microbial level before and after that sample were close to the reported average. The bacterial population identified in the potable water tank was more diverse than the bacterial population identified in the hygiene water tank. No correlation between the iodine concentration and bacterial population fluctuations was found during this test.

After the completion of WRT stage 7, 2 tanks, designated Storage Tank 3 (ST3) and Storage Tank 4 (ST4), were locked off when filled with recycled water from the PWP. The water was analyzed for microbial and chemical parameters from October 5, 1992 to April 5, 1993. The water was sampled 3 times per week for the first 4 weeks of testing and then twice per week until the water was depleted. The purpose of this Water Degradation Test was to investigate the long term storage effects on the quality of water reclaimed by the PWP.

The ST3 water samples averaged 0.36 CFU/100 mL < 1 CFU/100 mL in 20 out of 27 samples) for AEM counts. The average heterotrophic bacterial count in ST3 was 0.48 CFU/100 mL < 1 CFU/100 mL in 19 out of 27 samples). Results from the microbial counts of ST4 water samples

averaged 0.29 CFU/100 mL « 1 CFU/100 mL in 43 out of 48 samples) for AEM and 0.38 CFU/100mL « 1 CFU/10G mL in 44 out of 48 samples) for heterotrophic microorganisms. One sample with high microbial counts was reported from each of the storage tanks: 47 CFU/100 mL (ST3, AEM counts, test day 5) and > 80 CFU/100 mL (ST4, AEM counts, test day 157). The data before and after the microbial upset for the water sample from ST3 was < 1 CFU/100 mL. Results of a 1 L water sample, withdrawn from ST4 on the same day of the microbial upset (test day 157), contained a heterotrophic bacterial population of < 1 CFU/100 mL. It was concluded that both incidents of higher counts were a result of random microbial contamination during sampling and/or sample processing.

Staphylococcus spp. was reported 70% of the time microorganisms were identified in the processed water. Other microorganisms identified once during the test included *Pseudomonas saccharophila*, *Corynebacterium aquaticum*, *Rhodococcus spp.*, and *Nocardia spp.*

After the completion of WRT Stage 8, two of the potable tanks filled with reclaimed water were also isolated to assess the microbial quality of the water after an extended storage conditions. Results showed that over 101 and 183 days storage periods, no increase in the microbial population was observed. The length of the test for both tanks was based upon the availability of reclaimed water for sampling: when the water was depleted, the test was terminated. The average of the microbial assessment on CAE and R2A was 1 CFU/100 mL. The residual iodine level remained above specifications (1.0 mg/liter) for 73 and 92 days, after an initial concentration of 1.9 and 1.8 mg/L respectively. The TOC level, which was initially 0.7 mg/L, was measured at 0.44 mg/L on day 171, suggesting that limited consumption of TOC might have occurred.

It was concluded that it is possible to maintain the microbial water quality of a tank filled with low concentration of Total Organic Carbon (TOC) in iodinated recycled water for extended durations. A steady decrease in the concentration of iodine residual, as expected, was detected in both tanks.

5) Endotoxin test- Endotoxins are endogenous toxic components of the lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria. The endotoxins are liberated during cell growth or death. Although endotoxins from different bacterial species differ antigenically, they all produce similar physiological effects on the host (if infused or inhaled). The toxic activity of the LPS resides in its Lipid A moiety which is resistant to physical and chemical inactivation. Growth of Gram negative bacteria can result in a significant accumulation of endotoxins in a closed water recycling system.

During WRT Stage 8 selected water samples were sent to UAB and Boeing Laboratory for a Limulus Amebocyte Lysate (LAL) test (10). For comparison, samples from Birmingham city water (faucet water) and UAB's deionized and distilled water systems were also assayed for the presence of endotoxins. The LAL test is commonly used for the indirect detection of Gram-negative bacteria and/or quantitation of endotoxin contamination in a variety of substances. Values reported in this test were results of semi-quantitative comparisons made between known concentrations of endotoxin standard dilutions and the concentration detected in the samples. The combined wastewater (Port 124) contained levels of endotoxin of >2460 Endotoxin Units

per milliliter (EU/mL). This is consistent with the high viable microbial counts reported from this port. The levels of endotoxin in the processed water prior to storage (Ports 126 and 127) were reported as < 0.06 EU/mL. No change in the endotoxin level was detected in the stored processed water (Port 120) during testing. The level of endotoxin did not increase in the samples collected from the storage tank (Port 120) during extended storage of the processed water after Stage 8 (Water Degradation Test).

Analysis of samples from Birmingham city water assayed for the presence of endotoxin contained levels between 0.125 and 0.250 EU/mL. Laboratory deionized water contained levels between 0.060 and 0.125 EU/mL. Levels of endotoxin in samples from the glass distilled water system were reported between 0.250 and 0.500 EU/mL.

Samples from the WRT Stage 8 combined wastewater tank (Port 124) contained high levels of endotoxin. This observation correlated with the high viable bacterial counts reported for Port 124 (-109 CFU/100 mL). The concentration of endotoxin detected in samples from the processed water was reduced from > 103 EU/mL in the wastewater tank to < 0.06 EU/mL in the stored processed water. The processed water endotoxin level was also low compared with the levels detected in the city, distilled, and deionized water. This finding suggests that endotoxins are being removed or destroyed during water treatment. In addition, these data suggest that the viable bacterial counts from WRT processed water did not increase during extended storage after Stage 8 (Water Degradation Test). The absence of Gram-negative bacteria in the processed water was confirmed by the LAL test.

6) Analysis of Multifiltration beds- The potable water processor multifiltration beds were analyzed after they became saturated with contaminants during the WRT Stage 8 test. The inside of the multifiltration beds was exposed by aseptically cutting the stainless steel casing with a saw at predefined locations. After removing the resin material that was exposed to the cutting saw, between 2 to 7 grams of each material was placed in a sterile test tube containing a phosphate buffer solution.

Microbial Check Valve: The MCV is an iodinated anion exchange resin that was designed to impart 1 to 4 ppm of iodine residual to the influent water. Two layers of the inlet MCV (MCVIN) were sampled: MCVIN (Red) and MCVIN (Black). The first layer of the resin (MCVIN-Red) was the first also to receive the untreated wastewater. This resin had an orange-red color indicating the depletion of the resin's triiodide. The average microbial count of the depleted MCV was 1.3×10^7 CFU/g wet wt. No significant fluctuations from this average were reported in any of the 3 unibeds analyzed. Microorganisms identified included *Aeromonas caviae*, *Enterobacter spp.*, *Mycobacterium fortuitum*, *Serratia plymuthica*, and *Pseudomonas vesicularis*. The microbial count and microorganisms identified were similar to those organisms that were identified in the wastewater. The second layer of the resin (MCVIN-Black) was not discolored, exhibiting the characteristic black color of an unused resin. The average microbial count (1.9×10^2 CFU/g wet wt) was a 5 log reduction compared to the depleted MCV. Microorganisms identified included organisms not previously identified: *Bacillus sphaericus* and *Corynebacterium* group A.

Microbial Check Valve: The outlet MCV analysis demonstrated a microbial reduction of at least 2 log in Multifiltration bed #1. The results of Unibed #2 and #3 were reported as $< 1.3 \times 10^3$ CFU/g wet wt and $< 2.6 \times 10^1$ CFU/g wet wt, respectively. *Lonesia denitrificans* was the only organism identified in Multifiltration bed #1. No discoloration (red) of the resin due to triiodide depletion, such as the one noticed in the inlet MCV resin, was noted at the outlet MCV.

IRN-150: The IRN-150 media is a mix of two different resins: 1) IRN-77, a strongly acidic cation exchange resin primarily used to remove cations; and 2) IRN-78, a strongly basic anion exchange resin primarily used to remove anionic compounds. Since this resin occupies a large percentage of the multifiltration bed, it was sampled in two locations designated as IRN-150 (Early) and IRN-150 (Mid). Results from the IRN-150 (Early) averaged 1.4×10^4 CFU/g wet wt. An increase of 1 log was reported in the third unibed analyzed. Among the organisms identified in the first multifiltration bed were *Bacillus spp.*, *Lonesia denitrificans*, and *Nocardia spp.* *Pseudomonas syringae* and *Mycobacterium spp.* were identified in the second unibed; and *Comamonas testosteroni*, *Corynebacterium jeikeium*, and 3 species of *Enterobacter* were identified in the third multifiltration bed. These organisms were identified in the wastewater during testing.

The second location, IRN-150 (Mid), was sampled once (Unibed #1). The microbial load was reported to be 1.9×10^4 CFU/g wet wt, a 2 log reduction compared to the previous location. Organisms identified included *Bacillus spp.* and *Serratia plymuthica*.

580-26: The 580-26 multifiltration bed medium is activated carbon produced from coconut shell. Its primary function is to remove large organics. The average microbial count was 8.6×10^5 CFU/g wet wt, 1 log reduction than layer IRN-1S0 (Early) and 1 log increase if compared to the result from IRN-1S0 (Mid). Multifiltration #2 exhibited a slight microbial increase if compared to results from Multifiltration bed #1 and #3. Microorganisms identified included *Mycobacterium spp.*, *Alcaligenes faecalis*, and *Enterobacter spp.*

IRA-68: IRA-68 is a weakly basic anion exchange resin that primarily functions to remove organic acids. It was sampled once. Results were reported as 6.2×10^5 CFU/g wet wt. *Rhodococcus spp.* and unidentified acid-fast rod were identified.

Carbon Mix: The carbon mix media is composed of activated carbon produced from bituminous coal. Its primary function is to remove unidentified organics. The carbon mix also occupies a large percentage of the unibed. Sampling was divided in 3 locations designated as C-Mix (Early), C-Mix (Mid) and C-Mix (Late). The layer C-Mix (Early) had an average microbial load of 3.9×10^5 CFU/g wet wt. The average microbial load for C-Mix (Mid) and C-Mix (Late) media were 1.9×10^5 CFU/g wet wt and 1.9×10^5 CFU/g wet wt, respectively. These averages were similar to the microbial load in the previous layer. The organisms identified in the three C-Mix locations were similar including *Pseudomonas spp.*, *Bacillus spp.*, *Citrobacter spp.*, *Staphylococcus spp.*, and *Enterobacter spp.*

The microbial loads in most of the unibed media reflected a reduction from the feed wastewater. No significant microbial increase in the multifiltration bed media, which can be related to the position of the unibed, was observed.

Results from this study suggest that the inlet MCV resin might be providing limited microbial control inside the multifiltration beds, despite the plentiful availability of nutrients. The lower microbial load observed in Multifiltration bed #2 Carbon Mix (Early, Mid, and Late) could have resulted from a lower wastewater throughput for this bed during the test. Organisms surviving in the different media are the same organisms that were identified in the wastewater. The majority of these organisms were not recovered from the product water, indicating that the PWP is successfully reducing the initial microbial population to specified Space Station limits. Some of the bacteria identified in the multifiltration beds and in the processed water include *Staphylococcus spp.*, *Pseudomonas spp.*, and *Bacillus spp.* The presence of these organisms in the processed water can be attributed to contamination from external sources during sampling and/or processing.

7) Shower water/point of use (POU) assessment - During WRT, Stage 4/5, point-of-use water samples were analyzed from the shower in the EEF (Port 93), handwash faucet (Port 94), and potable dispenser faucet (Port 70). An increase in bacterial numbers between the hygiene storage tanks (clean. processed water) and the points-of-use EEF shower samples was observed. Microbial samples from Port 11, the port between the storage tank and the EEF shower (Port 93), averaged <1 CFU/100 mL during 21 test days. The possibility of back contamination may explain the microbial increase reported in Port 93. In addition, the nozzle used during this test was a hand-held shower nozzle that was in contact with the test subject's skin. This provided a constant and diverse microbiological population on the surface of the nozzle.

In order to compare the quality of the water used by test subjects while showering with municipally-treated water and recycled water, a study was performed to quantify and identify the microorganisms present in both types of water. Water samples from the shower located in the EEF (Port 93, recycled water) and the grey shower located at the west end of Building 4755 (Port 109, municipally-treated water) were collected. The samples were analyzed in parallel using CAE and R2A media. During the test the shower nozzle of the EEF (Port 93) was autoclaved on test day 4 (before batch 4 was collected) and test day 7, but on test day 11, hydrogen peroxide (3% H2O2) was used instead of heat sterilization. The microbial population from Port 93 fluctuated between 4.4×10^3 and 5.0×10^2 CFU/100 mL (the average was 5.0×10^2 CFU/100 ml). The microbial population of Port 93 maintained a <1 CFU/100 mL count 2 days after the shower nozzle was autoclaved or rinsed with H2O2. In contrast, the population from Port 109 fluctuated between 9.1×10^3 and 2.8×10^1 CFU/100 mL (the average was 4.0×10^3 CFU/100 mL). Microbial analyses of the sample water after the sterilization of the nozzle demonstrated that the majority of organisms recovered from Port 93 samples probably originated at the nozzle.

Microbial Characterization of Selected Home Showers- To compare the quality of reclaimed water used by test subjects while showering in the EEF, with municipally-treated water used in showers at home, samples from selected homes in north

Alabama were collected and analyzed on June 28, 1991. After flushing with several milliliters of sample water, the samples were collected in 500 mL bottles containing sodium thiosulfate and EDTA. Sample analyses consisted of enumeration of microorganisms by membrane filtration technique on R2A and CAE and identification of each different colony morphology on these media. The first sample was collected from a home in Decatur, the second sample was collected from a home in Huntsville, and the third sample was collected from an apartment complex in Madison, AL.

Viable counts were higher on R2A than on CAE and ranged from 2.9×10^2 to 1.2×10^4 CFU/100 mL. Predominant genera isolated included *Pseudomonas*, *Methylobacterium*, and *Bacillus*. The unidentified gram negative rods were most likely *Pseudomonas spp.* according to the fatty acid profiles of these isolates. The total counts on R2A from home showers were higher than the total counts on R2A from the apartment complex.

Wastewater: Obligate thermophilic bacteria were isolated from the shower outlet (*Bacillus licheniformis* and an unidentified irregular-shaped gram positive rod) and the combined waste tank (2 unidentified irregular-shaped gram positive rods).

Anaerobic sulfate reducers were isolated from humidity condensate, shower outlet, clothes wash outlet, and the combined waste tank. These organisms were tentatively identified as *Desulfovibrio spp.* Many species of the *Enterobacteriaceae* also gave positive reactions for sulfate reduction.

A few fecal streptococci were isolated from the shower outlet, combined waste tank, and the urine distillate. Species included *Streptococcus faecalis*, *Enterococcus avium*, and *Enterococcus malodoratus*.

Processed Water: Membrane filtration under aseptic conditions of liter quantities of recycled water indicated an average of 4 CFU/L of planktonic bacteria and fungi in recycled water.

Caulobacter, actinomycetes, pathogenic streptococci, mycobacteria, and legionellae were not isolated from ECLSS wastewater or recycled water samples using conventional plating methods.

Heterotrophic plate counts on R2A for ECLSS wastewater averaged 1.0×10^8 to 1.0×10^9 CFU/100 mL.

Levels of sporeformers, thermophiles, sulfate reducers, and fecal streptococci were isolated from ECLSS wastewater with differential and selective techniques. These organisms are present at significant numbers but had previously been missed due to the overgrowth of other microorganisms.

Viable pathogenic streptococci, mycobacteria, actinomycetes, and legionellae were not recovered in any ECLSS waters.

Membrane filtration under aseptic conditions of liter quantities of recycled water indicated an average of 4 CFU/L of planktonic bacteria and fungi in recycled water. This level of microbial contamination is less than the Space Station Freedom Water Quality Specification of 1 CFU/100 mL.

9) **Assimilable Organic Carbon-** A bioassay to measure the Assimilable Organic Carbon (AOC) concentration and to determine the bacterial regrowth potential in potable and hygiene processed (WRT Stage 4/5) water was performed by Dr. Carol Palmer of the University of California at Irvine. Nine clean water samples were analyzed, 5 from the potable water storage tank and 4 from the hygiene water storage tank. The AOC levels in the potable water samples were recorded as: 113 microg/L (7/12/91), 96 microg/L (7/15/91), 68 microg/L (7/24/91) 121 microg/L (7/30/91) and 116 microg/L (7/31/91). The AOC level did not cause an increase in the number of microbial isolates on R2A or CAE, as the culturable bacteria population was maintained at <1.0CFU/100mL.

In the hygiene water samples, the AOC levels steadily increased during the 2 week study from 103 µg/L to 150 µg/L. This increase in AOC levels could have been reflected in the microbial count increase from <1 CFU/100mL to 6 CFU/100mL on CAE reported by the laboratory.

IV. Conclusions

Results from the microbiological analyses performed during the WRT showed that it was possible to recycle water from different sources, including urine, and produce water that can exceed municipal produced potable water. This publication provides an overview of some of the microbiological analyses performed during the Space Station WRT program, tests that not only integrated several technologies with the goal of producing water that met NASA's potable water specifications, but also integrated humans, and therefore human flora. At the time these tests were performed, not much was known (or published) about the microbial composition of these types of wastewater. A document that will detail all the microbiological analyses, results and lessons learned from these tests is being prepared and will be published later this year. It is important to point out that design changes to the WRS have been implemented over the years and results discussed in this paper might be directly related to test configurations that were not chosen for the final, flight configuration. The data is presented for documentation purposes.

Information gained during the design and testing of a partially closed water recovery system for Space Station provided a basis for understanding the effects of microbial communities in the test environment. Although a significant amount of valuable information was gathered during ground testing, the uniqueness of a microgravity environment and the possibility of extending the

stay of humans in closed environments away from Earth pose new challenges and learning opportunities in the life support microbiology area. A balance needs to be found between keeping humans safe, equipment performing nominally for long periods of time and learning to live with the omnipresent microbial load. With increased knowledge of how microbes will behave in closed loop life support systems environments, technologies/system designs can be improved to minimize their negative/undesirable effects and maximize the positive/desirable effects. Even with the incorporation of the best life support design improvements, real-time microbial monitoring will be needed to assess the changes that will occur overtime in the microbial population. Reliable real-time monitoring on-board the spacecraft will be even more important as physical/chemical life support systems evolve, perhaps merging with biological-based systems and/or incorporating the use of *in-situ* resources during long-term missions far away from Earth.

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